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Prevalence of Staphylococci from Bovine Subclinical Mastitis and Assessment on Hygienic Practices in Dairy Farms, Eastern Ethiopia

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Abstract

A cross-sectional study was conducted from June 2022 to April 2023 to isolate of staphylococci in bovine subclinical mastitis, to establishing prevalence, to identify risk factors and to assess on hygienic handling practice of raw cow's milk in each selected dairy farm in Eastern Ethiopia. A semi-structured questionnaire survey and observation, California mastitis test (CMT), bacterial isolation and identification were conducted during the study. A total of 379 dairy cattle from dairy farms were screened for SCM by CMT. The prevalence of SCM in the study areas was 206(54.3%). Milk samples were collected from 206 cows free of clinical mastitis but positive in CMT. The prevalence of staphylococcal species among lactating cows with subclinical mastitis was 82.5%. The prevalence 92(44%) of Coagulase Negative Staphylococcus was higher than the Staphylococcus Aurous 78(37%). Among the risk factors studied, SCM due to staphylococci was significantly higher ($P < 0.05$) in older cows (94.3%) than younger (58.6%), in cows during late lactation (91.9%), in cows which gave more than 5 births (87%), in cross-breed cows (65.3%) than local breed cows (25.7%). Respondents with the age group ranging from 25 to 50 years and better educational level were acquainted that drinking raw milk is possible sources for staphylococci. This suggesting that more work on awareness creation is required in society with lower educational level compared to the society with better educational level. In addition, this is an opportunity to create awareness towards the prevention and control of zoonosis in actively involved group of society. Observational assessment of the milk handling practices in dairy farms showed that about (55.5%) of them were not washed their hands before milking. The study showed that the hygienic status of the udder and houses of lactating cows in the majority of dairy farms in the study area are poor, and the prevalence of subclinical mastitis and staphylococcal species are high. Based on results, creating the awareness, careful hygienic milking practice and regular health monitoring should be practiced to reduce prevalence of subclinical mastitis and staphylococcal infection.

Keywords: Hygiene; Lactating cow; Milk; Prevalence; Subclinical Mastitis; Staphylococci

Livestock is a significant national resource and an essential component of the agricultural production system in Ethiopia. The projected 65 million cattle and cows that comprise the main percentage of the nation's indigenous cattle are the largest livestock of any African nation (CSA, 2020). The bulks of the rural people, as well as a sizeable portion of the urban and peri-urban population, rely heavily on the milk generated from these animals as a food source. Mastitis, a condition of the mammary glands, is one of the many factors reducing milk supply, yet milk production frequently falls short of the nation's needs due to a variety of reasons (Berhanu et al., 2017).

The three types of udder injury caused by mastitis are clinical, subclinical, and chronic mastitis (Balemi et al., 2021). Mastitis is an inflammation of the mammary gland that is often caused by mastitis pathogens' adhesion, invasion, and colonization of the mammary gland. Subclinical mastitis is the most prevalent of these types and causes decreased milk supply without obvious clinical symptoms or defective milk (Pascu et al., 2022). Bovine mastitis is the most common endemic infectious illness affecting dairy cattle both domestically and globally. Mastitis results in decreased milk production, a change in milk composition, milk that is wasted, higher replacement costs, treatment costs, and veterinary services, which result in significant financial losses for dairy producers and the milk processing sector (Ejeta et al., 2022). Mastitis beings a multi-etiologic disease, about 137 infectious agents are known to cause mastitis in large domestic animals, of which bacteria are the major ones (Keane, 2019).

The bulk of mastitis cases in various dairy regions of the world are caused by staphylococci. The skin and mucosal surfaces of both humans and animals are commensal with Staphylococcus species (Lee et al., 2021). However, they have also been linked to human clinical disorders such as dermatitis, toxic shock syndrome, and food poisoning. Its strains are categorized into two groups, coagulase-positive staphylococci (CPS) and coagulase-negative staphylococci (CNS), based on their capacity to coagulate plasma (Fijalkowski et al., 2014).

S. aureus is primarily linked to subclinical and clinical mastitis in dairy calves among the coagulase-positive Staphylococcus species (Pyzik et al., 2019). *S. aureus* is the Staphylococcus species most frequently linked to preclinical and clinical mastitis in dairy calves among those that are coagulase-positive (Pyzik et al., 2019). Infections caused by S. aureus, a significant human and animal pathogen CPS, include septicemia, osteomyelitis, and superficial skin and soft tissue infections (Li et al., 2019). In the majority of nations with a dairy industry, Staphylococcus aureus is the most frequent germ that causes mastitis in cows. Staphylococcal food poisoning (SFP), a common food-borne illness that is typically not life-threatening, is brought on by ingesting staphylococcal enterotoxins (SEs), which are mostly released by CPS, particularly *S. aureus* (Fisher et al., 2018).

A few studies have been done in Ethiopia to estimate the prevalence of mastitis (Ananya et al., 2015), but very little is known about the disease of mastitis, especially subclinical mastitis. There is not enough research demonstrating how common mastitis and *S. aureus* from dairy cows are in Ethiopia. Examples include 44.9% *S. aureus* from Bishoftu Town (Birhanu et al., 2017) and 13.8% *S. aureus* and 11.7% S. epidermis from Holeta (Ayano et al., 2013), both from central Ethiopia, were reported in bovine sub-clinical mastitis. However, there were few studies on the incidence of *S. aureus* and CNS from sub-clinical mastitis in Eastern Ethiopia. Furthermore, control and prevention of such important diseases in the dairy sector require rigorous and systematic research and documentation of information on the status of the disease. Therefore, this study aimed to estimate the prevalence of staphylococci in cattle subclinical mastitis and assess milk hygienic handling practices in Eastern Ethiopia.

Materials and Methods

Description of the Study Area

A cross-sectional study was conducted in the selected dairy farms in Eastern Ethiopia including dairy farms in Harar, Dire Dawa, Babile and Jigjiga towns.

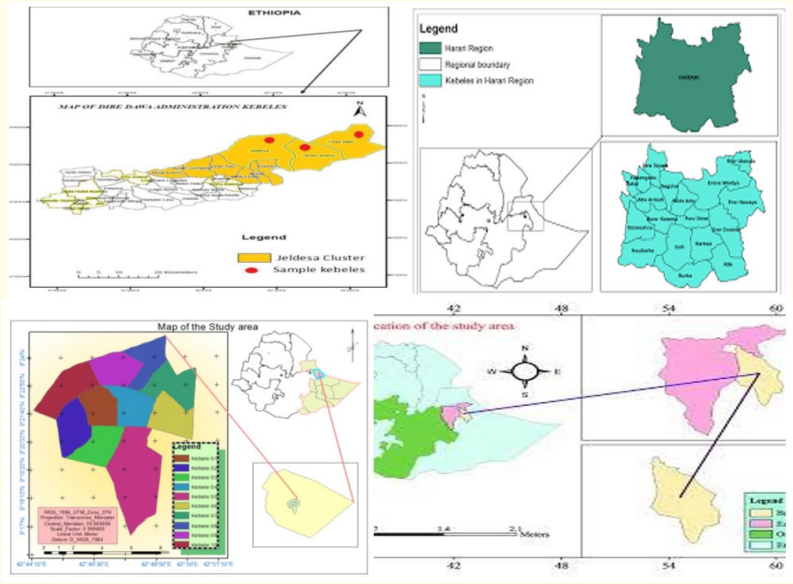
Harar, the capital of the Harari Region, is located at between 9° 18' 43" N latitude and 42° 07'23" E longitude at 515 km east of Addis Ababa, and lies in the south-western part of the region just off the southern edge of the southeastern plateau dividing the Great Rift Valley from the plains of the Ogaden lowlands. Administratively it is divided in to 19 town kebeles and 17 rural administrative units called peasant associations (PAs). Based on the available records, the mean annual daily temperature is 19.2 °C. The mean annual rainfall can be between 667 mm to 714.6 mm. Topographically, the Harari Region may be divided in to highlands (> 1400 masl) and lowlands/valley bottoms (<1400 m above from sea level) (Ishot, 2009).

Dire Dawa city administration is a city located in the eastern part of Ethiopia with in the valley surrounding eastern mountains of eastern Hararghe and at semi-desert of Somali region. Dire Dawa lies between 9° 27' and 9° 49' N latitudes and between 41° 38' and

42° 19'E longitudes, 515 Km from Addis Ababa, the capital city of Ethiopia. This area has hot climatic condition with annual minimum and maximum temperature of 18.2°C and 34°C, respectively and annual average rain fall of 676.3 mm. Out of the rural population about 4% are pastoralists engaged in livestock production as the only livelihood activity. The total livestock population in DDCA is estimated to be 219,323 (CSA, 2012).

Babile is a town in eastern Hararghe located 557Km east of the capital city of Addis Ababa. The average annual rainfall of Babile is 605mm, whereas the mean annual temperature is 26 °C. These areas are selected because; they have high camel slaughtering potential especially Dire Dawa and Babille.

Jigjiga is the capital of Ethiopian Somali Region which is the most eastern among the nine Regional States of the country. Jigjiga is the one among the nine Administrative Zones of the Region. It has an urban inhabitant's number of 621,210 (14%) of the population. It is located 615km from Addis Ababa in East direction with an altitude ranging from 1660 to 1850m above from sea level. It is geographically located at 8° 44'N longitude and 40° 22'E latitude with mean minimum and maximum temperatures of around 17 °C to 30 °C, respectively. According to National Meteorological Service Agency reports, the mean annual rain fall is 660mm and is bimodal in its distribution. The livestock population of the district is estimated to be 62,156 cattle, 100,516 sheep, 142,048 goats, 10,172 equines, 17,185 poultry, 12,825 camels and 695 bee hives. The majority (78.33%) of the farmers raises both crops and livestock, while 19.88% only grow crops and 1.79% raises only livestock (CSA, 2012).



Source: (Ethio. GIS).

Figure 1: Map of Study Area.

Study population

The study populations were those lactating local and crossbreeds cows from smallholder dairy farms in Metta distinct of Eastern Hararghe zone. All age lactating categories of cows and the number of parities were included. All of the animals were kept indoors and supplemented by products of brewery, molasses and hay and of the farms were intensive productions where dairy animals are kept indoors at zero grazing.

Study Design and Sampling Technique

A cross-sectional study was carried out from June 2022 to November 2022 apparently healthy lactating dairy cows. The sample size was calculated using the average 44.5% expected Staphylococcal prevalence from SCM calculated from of three reports (33.3% *S. aureus* and 66% *CPS* from Haramaya University (Alemu and Abraha, 2017) and 34.2% CNS from selected districts of Eastern Hararghe Zone (Zeryehun and Abera, 2017)) all from Eastern Ethiopia based on the formula given by Thrusfield (2008). The 95% confidence interval and 5% level of precision was also used. The total sample size using the formula given here below was 379.

$$n=\frac{1.96^2 p_{exp}(1-p_{exp})}{d^2}$$

Where n= required sample size, p= expected prevalence, and d=desired absolute precision.

Collection of Milk Samples

Approximately 10 mL of milk was collected from subclinical (CMT positive) mastitis cows into horizontally held sterile test tube after discarding the first 2-3 milking streams and transported to Hirna Regional Microbiology Laboratory for microbiological analysis in an ice box maintaining temperature at 4 0C±1 0c for further processing and analysis. Milk samples for bacteriology were collected aseptically. The udder and especially the teat were cleaned of dirt with a textile cloth moistened with distilled water. After that, the teat apex was cleaned with a cotton swab moistened with antiseptic solution. Samples are then stored in mobile refrigerator and transported to laboratory for further analysis.

Questionnaire Survey

Semi structured questioner were used to assess the management and hygienic practice of dairy farms. The farm owners, milking personnel and farm attendant from selected farms were face to face was interviewed on the way they handle and manage farm, milk and milk products. Generally, farm/animal owner, milk collector and farm attendant were interviewed while sampling. Consequently, hygienic practices employed in the farms such as house cleaning, udder cleaning, hand washing practices and milking utensils and collecting vessels (bucket) hygiene and other condition that affects the hygienic quality of raw milk were assessed. For questionnaire survey sample size was calculated using the formula given by (Arsham, 2005) N = 0.25/SE2, where: N = sample size, SE (standard error) = 5%. The required sample size for the questionnaire survey was 100.

California mastitis test (CMT)

In this study California mastitis test and bacterial isolation were conducted following standard procedures. California mastitis test (CMT) was carried out following the procedure described by NMC and Quinn et al. for screening subclinical mastitis. Briefly, a drop of the CMT reagent (4% NaOH in distilled water and 1% bromothymol blue) were put on the 4 cups of the CMT paddle into which equal amount of 5 ml of milk from the respective quarters of the cow were added and gently mixed by rotating the paddle in a horizontal plane for 20-30 seconds. The test result was interpreted based on the thickness of the gel formed by CMT reagent and milk mixture as 0 and trace for negative and +1, +2, and +3 for positive. Cows were considered positive for CMT, when at least one-quarter turned out positive.

Isolation and Identification

Bacterial culture and identification were conducted aseptically and collected by standard milk sampling techniques. The samples were inoculated aseptically onto sterile blood agar plates (BAP) enriched with 7% heparinized sheep blood, incubated at 37°C for 24-48 hrs under aerobic culture conditions, and examined for the presence of *Staphylococcus*. Colonies of *Staphylococcus* species were identified based on their morphological aspects (creamy, greyish, white, or yellow colonies) and hemolytic pattern on the surface of BAP. Presumed staphylococcal colonies were sub-cultured on nutrient agar plates (NAP) and incubated at 37°C for 24 to 48 hrs to get

pure culture. Pure cultures of a single colony type from the NAP were inoculated into nutrient slants and incubated at 37°C for 24-48 hrs under aerobic culture conditions; the pure isolates in the nutrient slant were preserved and maintained at 4°C for further analysis. The isolation and identification of *Staphylococcus* species were performed from pure isolates grown on NAP. The shape and arrangement of these colonies were detected after performing Gram staining. The catalase tests were conducted and the *Staphylococcus* species were assumed to be the colonies that produced gas bubbles. The colonies that were identified by Gram staining and catalase tests were sub-cultured on Mannitol Salt Agar (MSA) plates and incubated at 37°C examined after 24-48hrs for growth and change in the color of the medium. The presence of growth and change of pH in the media (red to yellow color) was regarded as confirmative identification of the salt-tolerant staphylococci. The fermentation of mannitol by *S. aureus* causes yellow discoloration of the medium. Colonies that develop weak or delayed yellow color after 24 hrs of incubation were regarded as *S. intermedius* and colonies that failed to produce any change on the medium were considered as *S. hyicus* and CNS.

Data Management and Analysis

The results data collected from the laboratory investigations were entered into Microsoft Excel and prepare for analysis. Descriptive statistics was performing using SPSS version 20 statistical.

Results

Questionnaire Survey and Observation

Socio Demographic Characteristics of Respondents

Out of 110 respondents toward educational status more of respondents 52(47.7%) were elementary, 24(21.8%) high school, 18(16.4%) illiterate and 16(14.5%) were college and above. Most of the respondents 76(69%) male and 34(30.9%) female were interviewed. According to the age of the respondents more of them 57(51.8%) were 25-50 years, 32(29%) 11-24 years and 29(19%) were 50-75 years (Table-1).

Factors	*Values*	*Frequency*	*Percentage (%)*
Educational status	Illiterate	18	16.4
	Elementary	52	47.3
	High school	24	21.8
	College and above	16	14.5
Sex	Male	76	69
	Female	34	30.9
Age	11-24 years	32	29
	25-50 years	57	51.8
	51-75 years	21	19

Table 1: Socio demographic characteristics of respondents.

Knowledge, Attitude and Hygienic Practices of the Respondent

From total of respondents 78(70.9%) were know about zoonosis disease and 88(80%) of respondents were know about the risk of contamination without any received of formal training relating to zoonotic disease and risk of contamination. Out of 110 respondents most of them 96(87.3%) were drinking raw milk due to carelessness and lack of knowledge, while 14(12.7%) of respondents were boiling the milk before consuming. More of respondents 58(52.7%) used cold water, 30(27.2%) used detergent and 22(20%) were used hot water for cleaning the milking bucket (Table-5).

Factors	*Values*	*Response*	*(%)*
Do you know zoonotic disease?	Yes	78	70.9
	No	32	29.9
Do you know contamination as risk?	Yes	88	80
	No	22	20
Do you Received training about zoonotic disease?	Yes	-	-
	No	110	100
Do you drinking raw milk?	Yes	96	87.3
	No	14	12.7
Why drinking raw milk?	Lack of knowledge	36	32.7
	Carelessness	60	54.5
Do you boiling milk?	Yes	14	12.7
	No	96	87.3
Clean milking bucket	Detergent	30	27.2
	Cold water	58	52.7
	Hot water	22	20
Container used for milking	Plastic	72	65.5
	Traditional	20	18.2
	Alumunium	18	16.4

Table 2: Knowledge, attitude, and hygienic practices of respondents.

Knowledge Towards on Zoonotic Disease with Socio Demographic Characteristics of Respondents

Majority of respondents (70.9%) were familiar with zoonotic disease in association with human health and know the possible source of staphylococci in consuming of raw cow milk. In this study the knowledge of respondents toward zoonotic disease was compered according to socio demographic characteristics. Out of 110 respondents toward their educational status, 18 of them were illiterate and only 6(33.3%) respondent know about zoonosis, 52 of them were elementary and 32(61.5%) of the respondents know zoonosis. All of (100%) the respondents those trained high school and college were know about the zoonosis. There was statistically significant difference between educational status with respondents were know about zoonosis disease (P=0.000) (Table 3).

Factors	*Values*	*Knowledgeable No (%)*	*Not knowledgeable No (%)*	*X^2(P-Values)*
Gender of respondents	Female	21(61.7%)	13(38.2%)	
	Male	57(75%)	19(25%)	1.995(0.158)
Total		78(70.9%)	32(29%)	
Age of respondents	11-24 years	22(68.7%)	10(31.2%)	
	25-50 years	43(75.4%)	14(24.5%)	
	51-75 years	13(61.9%)	8(38%)	1.465(0.481)
Total		78(70.9%)	32(29%)	
Educational status	Illiterate	6(33.3%)	12(66.7%)	
	Elementary	32(61.5%)	20(38.5%)	
	High school	24(100%)	0	
	College and above	16(100%)	0	30.944(0.000)
Total		78(70.9%)	32(29%)	

Table 3: Knowledge of the respondents regarding on the zoonotic disease.

Prevalence of Subclinical Mastitis due to Staphylococcal Species with Different Potential Risk Factors

In the present study parity, stage of lactation, age and breeds had significant association (P < 0.05) with the prevalence of subclinical mastitis due to staphylococci species. Cross breed cows are more affected than local (P < 0.05). Lactating cows with above 8 years age was highly infected with staphylococcal species, while those at the age interval of 5-8 years is less susceptible to staphylococcal species (P< 0.05), likewise prevalence of staphylococci was significantly high in cows with parity number more than 5 and less in those with parity l up to 3 (P < 0.05). Unlike early stage lactation, cows in late stage lactation Cows suffered most from staphylococcus species (P < 0.05) (Table 4).

Factors	*Values*	*Total number examined*	*No. of positive with SCM*	*(%)*	*X^2(P-value)*
Sample site	Dire Daw	136	92	67.6	16. 45(0.001)
	Harar	110	49	44.5	
	Jigjiga	62	33	53.2	
	Babile	71	32	45	
	Cross	105	27	25.7	
Breed	Local	273	179	65.3	48.014(0.000)
Age	3-5 years	116	68	58.6	94.571(0.000)
	6-8 years	175	55	31.4	
	>8 years	88	83	94.3	
Lactation Stage	1-3 months	128	52	40.6	76.238(0.000)
	4-6 months	152	63	41.4	
	>6 months	99	91	91.9	

Table 4: Prevalence of subclinical mastitis due to staphylococcus species with different potential factors.

Prevalence of Staphylococci Species among Lactating Cows at Study Area

The prevalence of staphylococci species among lactating cows with subclinical mastitis was 82.5%. In the current study high prevalence 92(44%) of Coagulase Negative Staphylococcus was observed than Staphylococcus Aureus 78(37%) (Table 5).

Staphylococci species	*Total number of isolated*	*Percentage (%)*
Staphylococcus Aureus	78	37
Coagulase Negative Staphylococcus	92	44
Total	170	82.5

Table 5: The prevalence of staphylococci (CPS and CNS) among lactating cows with subclinical mastitis.

Discussion

Respondents with the age group ranging from 25 to 50 years and better educational level were acquainted that drinking raw milk is possible sources for staphylococci. This suggesting that more work on awareness creation is required in society with lower educational level compared to the society with better educational level. In addition, this is an opportunity to create awareness towards the prevention and control of zoonosis in actively involved group of society.

The fact that drinking raw milk provides a chance for microorganisms, including staphylococci, to cause human infection. However, this study revealed that 87.3% respondents also consume raw milk. Thus, it is also the potential source of staphylococci and associated infection particularly for raw milk consumers indicated by occurrence of coagulase positive staphylococci 37% and coagulase negative staphylococcus 44%. Majority of respondents (87.3%) did not boiling raw milk while consuming duo to carelessness and absence of

received formal training in the community. But majority of respondents 70.9% know about zoonotic disease and 80% know contamination as risk through informal training and thought that staphylococci can be prevented through personal hygiene and cooked raw milk; this community knowledge would have paramount importance in staphylococci control but still need community training under sustainable condition.

Majority of respondents (70.9%) were familiar with zoonotic disease in association with human health and know the possible source of staphylococci in consuming of raw cow milk. In this study the knowledge of respondents toward zoonotic disease was compered according to socio demographic characteristics. Out of 110 respondents toward their educational status, 18 of them were illiterate and only 6(33.3%) respondent know about zoonosis, 52 of them were elementary and 32(61.5%) of the respondents know zoonosis. All of (100%) the respondents those trained high school and college were know about the zoonosis. There was statistically significant difference between educational status with respondents were know about zoonosis disease (P=0.000). But (87.3%) of respondents do not boiling the milk while consuming and drinking raw cow milk due to carelessness with knowledge of consuming raw milk cause source of staphylococci.

From 110 respondents, 76 of them were male and 57 (75.4%) of respondents know about zoonosis, and 34 of them were female and 21 (61.7%) of the respondents know zoonosis. Among 110 respondents towards their age, 32 of them were 11-24 years, and 22(68.7%) were know zoonosis, 57 of them were 25-50 years, and 43(75.4%) were know zoonosis and 21of them were 50-75 years, and 13(61.9%) were know about zoonotic disease. There was not statistically significant difference between sex and age with respondents were know about zoonotic disease (P>0.05).

Majority of respondents (52.7%) clean milking bucket by used only cold water and others (27.2%) used detergents while cleaning the milking bucket. Similarly, majority of respondents (65.5%) used plastic container for milking and milking transportation. However, *S. aureus* can enter milk through direct excretion from the udder of a cow with subclinical staphylococcal mastitis, as well as through contamination from the environment during raw milk handling and processing, posing a risk to consumers (Romero et al., 2018). Accordingly, poor hygienic practice during the milk handling may favor the multiplication of staphylococcal species.

In the present study the overall prevalence of bovine subclinical mastitis was 54.3%.The result was in agreement with (Romero et al., 2018) in Colombia, (Zeryehun and Abera, 2017) in Eastern Ethiopia, (Sumon et al., 2017) in Bangladesh and (Mpatswenemugabo et al., 2017) in Rwanda who reported prevalence of 55.2%, 51.8%, 51% and 50.4% respectively, and closely in agreement with the finding of 49.5% in New South Wales who reported (Plozza et al., 2011), 48% in Eastern Ethiopia who reported (Alemu and Abraha, 2017), 44.8% around Wolmera district who reported (Abdeta and Gemachisa, 2020) and 41.2% in Holata district who reported (Ayano et al., 2013). But the current study is in disagreement with findings of 71.4% in Eastern Ethiopia (Tafa et al., 2015), 67.9% in Aljeria (Sayeed et al., 2020), 38% in Egypt (Youssif et al., 2020), 28.34% in Assosa, 21.8% in Mecha district (Yimam et al., 2020) and 16.1% in Bishoftu (Birhanu et al., 2015). The differences in these studies could be attributed to the difference in the breed, management system, and the epidemiological status (Tafa et al., 2015).

Others study also showed that prevalence of 54.3% for subclinical mastitis that was much higher than the findings of 7.33% in Algeria (Sharma and Sindu, 2007), 16.1% in Bishoftu (Birhanu et al., 2017) and 28% in Algeria (Saidi et al., 2013) and much lower than the reports of 87.9% in Algeria (Kasozi et al., 2014), 86% in Indonesia (Qolbaini et al., 2014) and 85.33% in Nigeria (Shittu et al., 2012). Risk factors which influence the occurrence of subclinical mastitis were delineated as animal, pathogen, and environmental risk factors, which could contribute in the differences of subclinical mastitis prevalence (Abdeta and Gemachisa, 2020).

The current study showed that the difference prevalence of bovine subclinical mastitis was recorded among the samples of origins in the study area. High prevalence of subclinical mastitis 92(67.6%) recorded in Dire Dawa, followed by 33(53.2%) in Harar, 32(45%) in Jigjiga and the lowest prevalence 49(44.5%) was found in Babile town. There is statistically significant variation (p< 0.05) between the different sample of origins and positivity of subclinical mastitis. The difference may be due to greater experience in drying off, the potential effect of level of milking hygiene, herd size and cleanness, and the application of sanitary measures in these farms. This find-

ings disagreement with the report of (Zeryehun and Abera, 2017) who reported there were no statistically significant variation among the origins of the samples and positivity of subclinical mastitis.

In the current study the prevalence of staphylococcal species among lactating cows with subclinical mastitis was 82.5%. The prevalence 92(44%) of Coagulase Negative Staphylococcus was higher than the Staphylococcus Aureus 78(37%). This finding was agreement with the reports of (Murethi and Njugun, 2016) who reported 35.5% Staphylococcus Aureus in Kenya, 33.3% in Ethiopia (Alemu and Abraha, 2017) and 40% in Algeria (Saidi et al., 2013). But the finding of the present study lower than the reports of Mpatswenumugabo et al., (2017), Birhanu et al., (2017) and Youssif et al., (2020) those reported 51.5% of coagulase negative staphylococcus in Rwanda, 44.9% of staphylococcus Aureus in Ethiopia and 66.7% of staphylococcus Aureus in Egypt respectively, and much higher than 10% of coagulase negative staphylococcu in Bangladesh (Sumon et al., 2017), 13.8% of staphylococcus aureus in Holeta (Ayano et al., 2013), 18.33% of staphylococcus aureus in Bangladesh (Sumon et al., 2017), 20.6% of staphylococcus aureus in Rwanda (Mpatswenu et al., 2017) and 34.2% of coagulase negative staphylococcus in Ethiopia (Zeryehun and Abera, 2017).

The study showed that there were significant statistical associations ($p < 0.05$) between the prevalence of subclinical mastitis with the age and parity of animals, where risk of mastitis increases with age and parity number. The present result was in agreement with the observation of (Tafa et al., 2015), who stated that parity and age are significantly associated with infection rates. Similarly (Birhanu et al., 2017) in Bishoftu town and (Zeryehun and Abera, 2017) in Eastern Ethiopia have reported that cows with many number of cows were with higher prevalence of mastitis, while similar to the finding of the current study, prevalence of subclinical mastitis was reported to increase with age in study conducted in Colombia (Romero et al., 2018) and Nigeria (Shittu et al., 2012). The higher prevalence in older cows in the present study might be due to prolonged period of exposure to the infecting organisms and predisposing factors like stress of lactation which favor dilation of teat canal due to repeat milking thereby facilitating the entry of pathogens into the teat canal to cause subclinical intramammary infection (Sumon et al., 2017).

Prevalence of subclinical mastitis due to staphylococcal species was significantly higher (P <0.05) in cross breed cows than the local breeds which is agreement with the report of (Tafa et al., 2015) who reported high prevalence of mastitis in Jersey breed than local zebu cattle. This could be due to anatomically large pendulous udder of cross breed cows which can be easily exposed to environmental injuries which in turn predispose cows to suffer from mastitis. In this study, prevalence of subclinical mastitis was significantly higher (P<0.05) in late lactation as compared to early and mid-lactation, likewise Mureithi et al., (2016) and Mpatswenu et al., (2017) states that increased prevalence of mastitis was encountered as lactation stage advance. On the contrary, Youssif et al., (2020) and Saidi et al., (2013) reported that cows at early stage of lactation are more susceptible to subclinical mastitis.

Conclusion and Recommendations

The present study recorded an overall prevalence of subclinical mastitis was high in the study areas (54.3%) which might entail that mastitis was a major health problem of dairy cows which undoubtedly will have drawback on productivity of dairy industry and hence warrants serious attention. The result of study also display that pathogenic Staphylococcal species are the major bacteria along with other environmental bacteria to be associated with sub clinical mastitis. In identified staphylococcal species, the prevalence of coagulase negative Staphylococcus (44%) was higher than the coagulase positive staphylococcus (37%). The high prevalence of staphylococcal infection detected in dairy cows has public health concern since coagulase positive staphylococcus bacteria's are capable of producing heat-stable enterotoxins, which might cause staphylococcal food poisoning outbreaks when ingested by humans in sufficient quantities. This could be an indicator of poor hygienic practices and absence of regular health monitoring of dairy animals. Based on the above conclusion the following recommendations were forwarded:

- Regular screening for the detection of subclinical mastitis and proper treatment of cows during dry and lactation period should be practiced.
- Careful hygienic milking practice and regular health monitoring should be practiced to reduce reservoir of infection and contamination of the rest of the herd.

- Further research to identify species and strains of coagulase positive and coagulase negative staphylococcus have paramount significance to reduce and prevent the pathogen effect on dairy industry.

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